1: Chem Phys Lipids. 1994 Feb;69(2):137-50. Related Articles, Links

Entrez PubMed

PubMed Services

Related Resources

Characterization of the steady-state and dynamic fluorescence properties of the potential-sensitive dye bis-(1,3-dibutylbarbituric acid)trimethine oxonol (Dibac4(3)) in model systems and cells.

Epps DE, Wolfe ML, Groppi V.

Upjohn Company, Kalamazoo, MI 49001-0199.

The steady-state and dynamic fluorescence properties of the membrane potential-sensitive bis-oxonol dye Dibac4(3) were characterized in vitro using model ligand systems and in vivo in A10 smooth muscle cells by fluorescence microscopy in conjunction with the ACAS imaging system. In the latter system the dye responds to potassium ion-induced jumps in membrane potential with changes in its fluorescence intensity, which follow pseudo-first-order kinetics. The relationship between the magnitude of the changes and the corresponding rate constants excludes the possibility that a simple, one-step equilibrium between extracellular and cytoplasmic dye would be sufficient to account for this phenomenon. The necessity of invoking an additional step suggested that the redistribution of the free dye between the cytoplasm and the exocellular medium is rapid and that the slow step associated with the fluorescence changes may be the interaction of the dye with proteins in the cytoplasm, along the lines proposed by Brauner et al. (Biochim. Biophys. Acta 771 (1984), 208, 216). The interaction of the dye with BSA and with egg lecithin SUVs was studied as a model for the in vivo phenomenon. The dependence of fluorescence intensity changes on the concentrations of the reagents shows the formation of a reversible dye/albumin complex with a 2/1-stoichiometry, with Kd = 0.03 +/- 0.01 microM and a reversible adsorption to the SUVs with Kd 0.45 +/- 0.08 microM. The fluorescence lifetime of the dye in solution, < 100 ps, results in a high solution steady-state anisotropy. The latter decreases considerably upon binding to BSA, SUVs and A10 cells concomitant with a large increase in the lifetime. With such a short lifetime of the free dye, selective gating of the excitation source or the photodetector should eliminate the high background of the unbound dye and thereby enhance the sensitivity of the system.

PMID: 8181103 [PubMed - indexed for MEDLINE]